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−116A and *K BCHE* gene variants associated with obesity and hypertriglyceridemia in adolescents from Southern Brazil

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ABSTRACT

Butyrylcholinesterase (BChE) has been associated to body mass index (BMI), weight, cholesterol and triglyceride levels. *−116A* (rs1126680) and *K* (*A539T*, *1615A*, rs1803274) *BCHE* gene variants had previously been associated to BChE activity, weight and BMI variance in adults. The present study examined *−116A* and *K* variants, BChE activity, anthropometric and biochemical variables associated with obesity in adolescents (120 obese and 150 non-obese from Curitiba, Brazil). Both *−116A* and *K* variants were found with significantly lower frequencies ($p < 0.05$) in obese adolescents when compared with non-obese adolescents and with the general population. Mean BChE activity (KU/L) was significantly higher in obese adolescents when compared with non-obese adolescents and with the general population. Analyzing only the obese adolescents, it was found that carriers of the *−116A* variant showed lower BChE activity and higher triglyceride levels than homozygotes for the usual allele. Indeed, obese carriers of the *−116A* variant had triglyceride levels considered high according to reference values for serum triglycerides in Brazilian adolescents. These results show: (1) a protective effect of *−116A* and *K* variants on juvenile obesity risk, suggesting a role for the *BCHE* gene on juvenile onset obesity different from that observed on adult onset obesity and (2) an association of the *−116A* variant with hypertriglyceridemia in obese adolescents probably because of its effect on lowering BChE activity and consequently diminishing the enzyme capability of maintaining homeostasis on lipid metabolism during the metabolic stress caused by obesity.

1. Introduction

Butyrylcholinesterase (BChE; EC 3.1.1.8) is a serine hydrolase which is related to lipid metabolism and has been associated to BMI, waist–hip ratio, waist circumference, weight, cholesterol and triglyceride levels [1–6].

The most common variant of the coding region of the *BCHE* gene (3q26.1-q26.2) is the *K* variant (SNP: G/A; rs1803274; p.A539T; 1615 nt) located at exon 4, found with a frequency of 18.4% in Euro-Brazilians [7] and which has been associated with higher BMI variance (2). The non-codifying exon 1 presents a SNP at −116 nt (SNP: G/A; rs1126680), being the *−116A* preferentially found in cis with the *K* variant [8]. The *−116A* variant was first identified with a frequency of 8.0% [8] and was associated with lower BChE activity (4). Although the *K* variant was originally associated to lower BChE activity, it was shown that this variant alone is not associated with decreased BChE activity, being the *−116A* variant necessary for this decrease [4]. In a previous study of our research group [4], although we did not find differences on frequencies of *−116A* and *K* variants of *BCHE* gene between obese and non-obese adults, we did find that samples with different BMI distributions present different relationships between *BCHE* genotypes and BMI.

Considering linkage disequilibrium between these variants located at exons 1 and 4 of the *BCHE* gene, as well as their known relation with obesity, this study compared genotypes of these two sites in relation to BChE activity, anthropometric and biochemical variables associated with obesity in obese and in non-obese adolescents from Curitiba, Brazil.

2. Materials and methods

2.1. Samples

Analyses concerning *−116A* and *K* variants of the *BCHE* gene, and BChE activity used 120 obese adolescents (mean age = 12.8 y ± 0.15 y; 50 girls and 70 boys) and 150 non-obese adolescents (mean

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Table 1
Genotype and allele frequencies of *−116A* and *K* variants and comparisons between obese and non-obese adolescents by χ^2.

−116A variant	N[a]	Genotype frequencies[b]			Allele frequencies	
		GG (%)	GA (%)	AA (%)	G (%)	A (%)
Obese	120	93.3	6.7	0	96.7 ± 1.2	3.3 ± 1.2
Non-obese	144	81.9	18.1	0	91.0 ± 1.7	9.0 ± 1.7
$\chi^2_{(1)}$ (p)		**6.59 (0.01)**			**6.13 (0.01)**	
K variant	N[a]	Genotype frequencies[c]			Allele frequencies	
		GG (%)	GA (%)	AA (%)	G (%)	A (%)
Obese	114	81.6	17.5	0.9	90.8 ± 1.9	9.2 ± 1.9
Non-obese	150	72.5	23.5	4.0	84.2 ± 2.1	15.8 ± 2.1
$\chi^2_{(1)}$ (p)		4.79 (0.09)			**4.45 (0.03)**	

[a] The difference on number of individuals is due to lack of amplification of samples.
[b] GG = homozygotes for −116G allele; AA = homozygotes for *−116A* allele.
[c] GG = homozygotes for 1615G (usual) allele; AA = homozygotes for 1615A (K) allele.

age = 13.7 y ± 0.25 y; 50 girls and 100 boys). Both, obese and non-obese were Euro–Brazilians. Weight was measured to the nearest 0.1 kg and stature was measured to the nearest 0.1 cm. The BMI was standardized to *z*-score (BMI *z*-score) through the conversion of BMI subtracted the value corresponding to the 50th percentile of BMI and divided by the population standard deviation, as charts released by the CDC (*Center for Disease Control and Prevention)*, for every age and each gender [9]. Adolescents were included on this obese sample according to the following criteria: BMI ⩾ 95th percentile and stable weight for at least two months. Individuals submitted to anorexigenic drugs were excluded from the study. The anthropometric variables evaluated on obese adolescents were: weight; height; *z*-score, waist circumference (WC) and waist/height ratio (WHR). The study was approved by the Ethics Committee of the Federal University of Paraná.

2.2. DNA and plasma analysis

Mini Kit DNAAmp (Quiagen) was used for DNA extraction from plasma of 83 obese individuals. After DNA extraction, samples were dried out and resuspended in 50 μl of ultrapure water to achieve 20 ng/μl final concentration. For the remaining 37 obese samples and 150 non-obese samples, DNA was extracted by a salting out method [10] and diluted to 20 ng/μl final concentration.

Genotyping of exon 1 and exon 4 variants (rs1126680 and rs1803274, respectively) were achieved using *TaqMan SNP* Genotyping Assay (*Applied Biosystems*). Reactions were performed in Mastercycler Realplex 2 according to the following steps: (1) 50 °C/2 min.; (2) 95 °C/10 min.; (3) repeat 50 times 95 °C/15 s. interspersed by 62 °C/min.

Plasma BChE activity was measured using propionylthiocholine as substrate at 25 °C [11]. Glucose, triglycerides (TG), total cholesterol (TC) and fractions were measured by automated standard methods.

2.3. Statistical analysis

Frequency distributions, means ± S.E, variances and t-test were calculated using Statistica for Windows (StatSoft, Inc.,1996; http://www.statsoft.com). χ^2-tests were performed using Clump [12]. SPSS for Windows (SPSS Inc., 2004) was used for multiple regression analysis.

3. Results and discussion

Table 1 summarizes the results for allele and genotype frequencies in obese and non obese adolescents and comparisons between groups. *K* variant frequencies are significantly lower in obese than in non-obese adolescents ($\chi^2 = 4.45$; $p = 0.03$), as well as in obese adolescents when compared with those found in adult blood donors from Curitiba, PR (7; $\chi^2 = 5.47$; $p = 0.02$). Analyses of *−116A* allele frequency revealed that it is significantly lower in obese than in non-obese ($\chi^2 = 6.13$; $p = 0.01$). The *−116A* allele frequencies are also lower in obese adolescents than in a blood donor sample from Curitiba, PR (7; $\chi^2 = 6.77$; $p = 0.009$). Considering that the present study found lower frequencies for *−116A* and *K* variants in obese adolescents, it may suggest a protective effect of these variants on juvenile obesity risk, and a role for the *BCHE* gene on juvenile onset obesity stronger and different from that observed on adult onset obesity. An early onset complex disease, such as juvenile obesity, may have a distinct (stronger) genetic effect than that of late onset, such as adult onset obesity which may be highly influenced by environmental factors (nutrition and physical activity).

Comparing mean BChE activity between genotypes of the *−116A* variant site, it was found, as already shown for obese adults [4], that mean BChE activity in obese adolescents is lower in *−116A* carriers than homozygotes for *−116G* allele (3.9 ± 0.7 e 7.7 ± 0.3, respectively; $t = 2.9$, $p = 0.02$).

As shown in Table 2, the comparisons regarding biochemical and anthropometric variables between genotypes of obese adolescents showed only one significant difference: mean triglyceride level is higher in *−116A* carriers than in *-116G/-116G* homozygotes. Logistic regression analysis was performed to verify if the variables sex, age, *z*-score, WHR, glucose, *−116A* and *K* variants are independent risk factors for increasing triglycerides level on obese adolescents. The results revealed that only the *−116A* variant was a significant independent factor (B ± SE = −22.7 ± 15.191; $p = 0.0001$). It is also noteworthy that the mean triglyceride level of obese bearing the *–116A* variant is high according to the reference values proposed for this age group (ideal values ⩽130 mg/dL, high values >130; [13]. Previous studies [14–16] had proposed that the role of BChE on lipid metabolism could be the hydrolysis of choline esters, which are products of the free fatty acids metabolism and liver lipogenesis.

Table 2
Means ± standard error (SE) of anthropometric and biochemical variables in obese adolescents classified according to genotypes of −116 and 1615 sites of *BCHE gene*.

Variables	−116 site			1615 site[a]		
	−116G/−116A	*−116G/−116G*	*p*	*UK*	*UU*	*p*[**]
Z-score	3.34 ± 1.31	3.49 ± 1.11	0.79	29.6 ± 0.8	29.8 ± 0.6	0.89
WHR	0.61 ± 0.03	0,61 ± 0,01	0.85	0.61 ± 0.01	0.61 ± 0.08	0.96
Total cholesterol	176.1 ± 11.7	156.4 ± 3,2	0.12	165.5 ± 6,8	156.8 ± 3.7	0.29
LDL cholesterol	94.3 ± 9.8	89,6 ± 2,7	0.7	95.9 ± 5.4	88.4 ± 3.1	0.27
HDL cholesterol	45.9 ± 2.8	43.8 ± 0.8	0.52	44.5 ± 1.8	44.0 ± 0.9	0.84
Tryglycerides	244.0 ± 26.0	110,0 ± 10.7	0.00002	139.6 ± 20,1	117.5 ± 7.4	0.22
Glucose	88.6 ± 2.8	89.9 ± 0.9	0.68	91.0 ± 1.9	89.5 ± 1.0	0.51

[a] KK genotype was excluded from analysis ($n = 1$).
[**] *p* values for *t*-test.

Considering that, an increased availability and/or liver flow of free fatty acid or an increase of lipogenesis from carbohydrates, could lead to two effects: hyperlipidemia and increased BChE activity. The *−116A* variant might be associated with hypertriglyceridemia in obese adolescents because of its effect on lowering BChE activity and consequently diminishing the enzyme capability of maintaining homeostasis on lipid metabolism during metabolic stress caused by obesity.

Conflict of interest

The author declare that there are no conflicts of interest.

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